

Charge Exchange and Chemical Structure at Protein-Semiconductor Interfaces

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This project examines the electronic properties of semiconductor interfaces with biological molecules – an emerging research area whose exploration could catalyze innovative advances in biomedicine, surface science, and sensor electronics.

Objective: Determine and control charge transfer of individual protein molecules with SiO₂ on Si.

Approach: Use electronic gain of field effect transistor (FET) gate to assess charge transfer between biomolecule and surface. Use high-affinity Streptavidin-Biotin interaction as receptor-target molecule system to isolate charge transfer and AFM to measure bound molecules, yielding charge transfer per biomolecule.

Results: Changes in FET drain-source current correspond to $\sim 10^9$ charges cm⁻² transferred. Charge transfer $> 0.1 e^-$ per biomolecule.

Significance: From I-V transistor curves, we have determined charge transfer per biomolecule. The large electrical response to even small charge transfer demonstrates great potential for high sensitivity and selective biosensors.

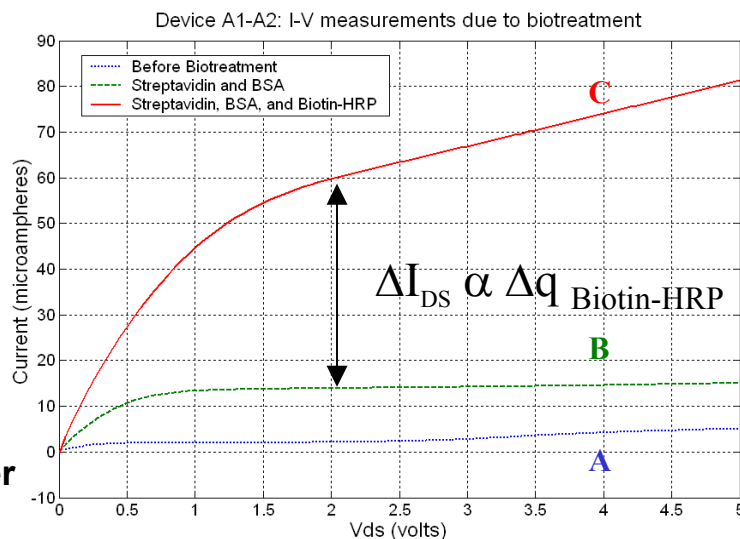
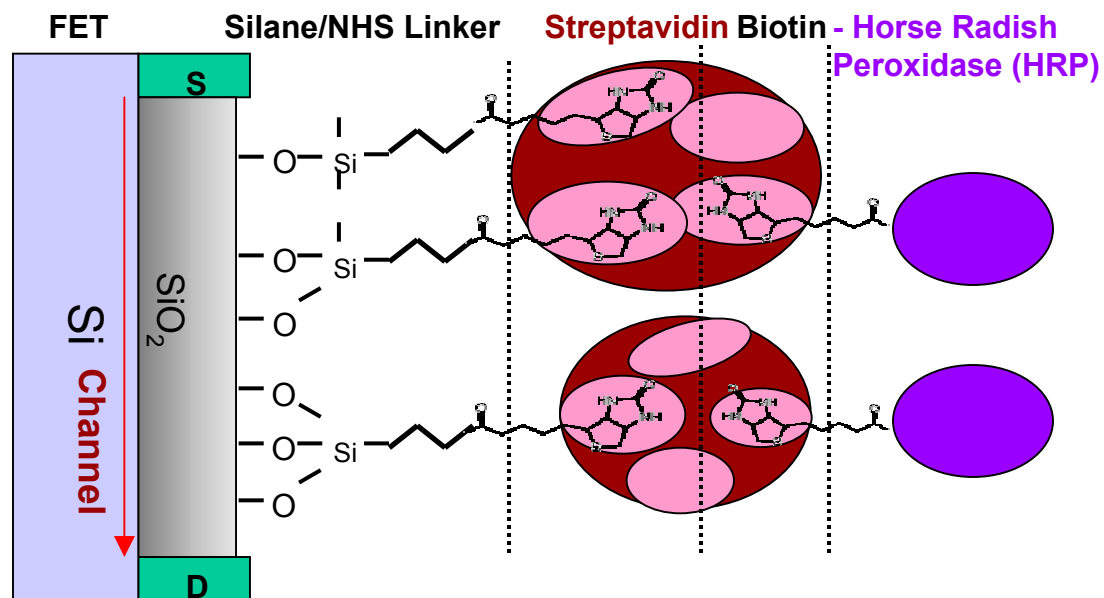


Fig 1. FET drain-source current vs. voltage measured for:

A. bare SiO₂ gate
B. SiO₂ + blocker (BSA) & Streptavidin receptor (SA)
C. SiO₂ + blocker (BSA) & Streptavidin (SA) receptor bound to target biomolecule (Biotin-HRP) showing large current increases due to receptor-target bonding that creates gate bias.



This project examines the electronic properties of semiconductor interfaces with biological molecules – an emerging research area whose exploration could catalyze innovative advances in biomedicine, surface science, and sensor electronics. Biological molecules exhibit charge transfer at semiconductor interfaces that change as the adsorbed molecules link to other biological species. Charge transfer at biological interfaces is a pervasive phenomenon, yet the properties of biological interfaces with electronic materials are virtually unexplored. Understanding and control of the bio/semiconductor interface along with its electronic, chemical, and biological activity can also enable advances in biosensors for monitoring biological functions in vivo, detecting pathogens and other biologically active species. The goals of this project is to understand, optimize, and control these bioelectronic phenomena.

Key objectives of this project are the correlation of the charge exchange measured at the Si/SiO₂ interface with the known charge properties of the conjugated biomolecule or protein, with the morphology of the Si/SiO₂ layer, and with the protein's strength of bonding with this layer. Furthermore, this knowledge could help extend the design of biological sensors if semiconductor transistors, which amplify the charge transfer associated with the adsorption process, can be extended with the design of biologically-active and specific charge transfer sites.

Initial Studies: We want to increase density and strength of chemical bonding while retaining high charge transfer. The challenge is that surface modification can introduce localized states that introduce dipoles that can screen electric fields that would otherwise modulate current in channel of transistor.

For example, roughening the surface introduces asperities and dangling bonds that increase surface coverage but could also introduce localized electronic states. AFM or FIB patterned surfaces show evidence for adsorption with roughness comparable to the 15 nm diameter of a Streptavidin molecule after protein application. FET and AFM measurements will tell if such roughening alters charge transfer per molecule. Furthermore, 50 nm wide trenches exhibit a decrease in depth by 11 nm or more, indicating a partial filling in of the trench by the Streptavidin molecule. A virtue of this “pocket” technique is that counterions can not collect around the Streptavidin and screen out charge transfer with the SiO₂ substrate.

Another approach has been to introduce an organized set of attachment points for the Streptavidin with a derivatized silane molecule (N-hydroxylsuccinimide) linked to a biotin molecule, which then links to the Streptavidin. In turn, this Streptavidin (**red**) serves as the receptor for a **biotin-HRP (horse radish peroxidase, dark purple)** target molecule. This approach has the advantage of introducing an

organized set of strong covalent bonds to the host substrate - versus the weak physisorption with simple Streptavidin exposure to the substrate. At the same time, it does not introduce any significant distance between the Streptavidin and the surface, thereby minimizing any screening of the charge transfer by neighboring ions in solution. Streptavidin has **four biotin-binding pockets (light purple)**. Two or one may be attached to the biotin on the surface, with the remaining 2 or 3 available to bind the biotin analyte. The biotin to streptavidin bond is one of the strongest non-covalent bonds known, so the combination of the covalent bond of the biotin to the silane (to the SiO₂ surface) and the streptavidin-biotin bond is much stronger than a streptavidin adsorption to the SiO₂ surface directly.

New Analytic Tool: We have developed a biosensor based on a modified Metal Oxide Semiconductor Field Effect Transistor (MOSFET) to study charge exchange at a protein/semiconductor interface. We used Streptavidin bound to Si/SiO₂ and the small molecule biotin as a model receptor-target testbed. Critical to maximizing the sensitivity to charge transfer are both a high surface density of receptor (Streptavidin) sites and a high charge transfer per receptor site.

With the metal gate of a traditional MOSFET removed to expose the underlying SiO₂ interface (fig. 1), proteins can be applied and charge transfer due to affinity binding events can control the sensor's current output. Experiments to compare the physical, morphology-induced, and chemically-assisted adsorption techniques for applying proteins and improving surface coverage are compared using fluorescence microscopy (FM), enzyme linked immunosorbent assays (ELISA), and dry/wet atomic force microscopy (AFM). Electrical tests of modified FETs successfully demonstrate that devices respond to charge transfers induced by Streptavidin/Biotin-HRP binding events (fig.2) and are used to evaluate the effective charge transfer at the oxide gate as perceived by the FET – on the order of 10⁹ charges/cm². Preliminary AFM results show the change in surface morphology consistent with adsorption of Streptavidin (diameter 15 nm) at a density of less than 1 per 100 nm square. The resultant charge transfer is therefore 10⁹ charges cm⁻² / <10¹⁰ cm⁻² charge transfer sites = > 0.1 electron/site.

Our effort now centers on producing high densities of individual charge sites rather than the widely spaced “clumps” shown in the AFM. Not shown are nanoscale physical patterning of the SiO₂ interface using a Ga focused ion beam that reveals a weak preferential binding of proteins to surfaces with dangling bond structures (e.g., step edges, rough surfaces). Also not shown are chemical modification of the surface using self-assembled monolayers (SAMs), in this case short-chain silane “linker” molecules that supports an organized seed layer of biotin that is covalently bound to the surface and provides sites for additional Streptavidin-Biotin binding and charge transfer. We are now preparing new FET structures to measure both the charge transfer and the Streptavidin binding site density on the same chemically-modified surface in vitro.

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Education: This project supported the undergraduate research for B.R. Cipriany and graduate research for M.T. Keener. It has attracted the interest and commitment of 3 additional undergraduates to the interdisciplinary studies of biotechnology with electrical and biomedical engineering.



Left to Right:
B.R. Cipriany, Advisor Dr. Leonard Brillson, and M.T. Keener in Washington D.C. for NSF presentation

Outreach: The ability to detect low concentration organic and biomolecular compounds attracted the interests of industry, medical, and government representatives during Ohio State's annual Denman Undergraduate Research Forum – sharing the possibilities for stable, sensitive, and selective, compact sensors for a variety of applications.



B.R. Cipriany explains sensor concept to a representative of a local electric utility